FSUM 10302
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Robert A. Holton et al.
Serial No. 09/063,477
Filed April 20, 1998
For PROCESS FOR THE SELECTIVE DERIVATIZATION OF TAXANES
Examiner Ba K. Trinh

TO THE COMMISSIONER OF PATENTS AND TRADEMARKS

SIR:

DECLARATION OF ZHUMING ZHANG UNDER 37 C.F.R. 1.608(b)

I, Zhuming Zhang, declare and state as follows:

1. I was a post doctorate student at Florida State University in Tallahassee, Florida. At the time of the invention, I was conducting research in Dr. Robert Holton's laboratory in the area of Synthetic Organic, Biorganic, and Organometallic Chemistry.

2. I am an inventor of "Process For The Selective Derivatization Of Taxanes" of application no. 09/063,477.

3. I conducted the "Attempt to protect C(7)OH by cbz" experiment shown on laboratory notebook page 45 before May 21, 1997 (Exhibit A). I added 53 mg of dibenzyl dicarbonate to 3 mg of 10-deacetyl baccatin III in tetrahydrofuran solvent at room temperature and allowed the mixture to react overnight. A proton NMR of the final reaction mixture revealed that 10-benzoyloxy baccatin III had been produced in greater than 90% yield.

4. I conducted the "Attempt to protect C(10) H by (CH_3CO)-O" experiment shown on laboratory notebook page 49 before May 21, 1997 (Exhibit B). I added 10 microliters of acetic anhydride to 3 mg of 10-deacetyl baccatin III in tetrahydrofuran solvent at room temperature. I added an additional 100 microliters of acetic anhydride and allowed the mixture to react overnight. A proton NMR of the final reaction mixture revealed that baccatin III had been produced in greater than 80% yield.

5. I conducted the "Generation of baccatin III from 10 DAB" experiment shown on laboratory notebook page 67 before May 21, 1997 (Exhibit C). I added 1 ml of acetic anhydride to 14 mg of 10-deacetyl baccatin III and 3.5 mg $ZnCl_2$ in tetrahydrofuran solvent at room

temperature. A proton NMR of the final reaction mixture revealed that baccatin III was a major product of the reaction.

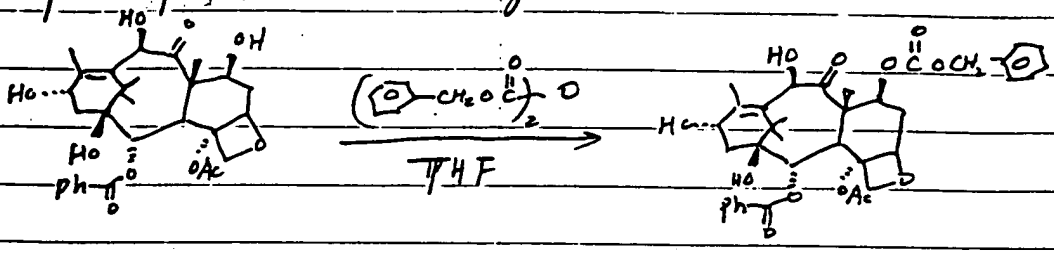
6. I authored the experimental descriptions found on laboratory notebook pages 45, 49 and 67 before May 21, 1997.

7. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

7/27/00
Date

Zhuming Zhang
Zhuming Zhang

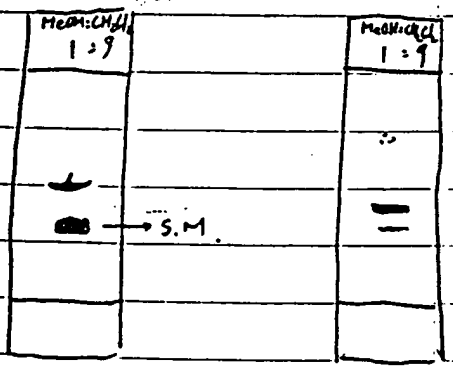
Attempt to protect C(7) OH by Cbz



Materials used	FW	AMT
(1) 10 DAB	544	3 mg (0.00551 mmol)
(2) dibenzyl dicarbonate (97%)	286.29	53 mg (3 eq., 0.484 mmol)
(3) THF		0.5 mL

Procedure: same as Page 44, except THF was used instead of CH_2Cl_2

The rxn proceeded smoothly, TLC indicated the formation of product. The rxn was left for overnight.

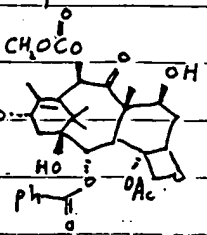


0.5 h overnight.

This rxn almost go to completion, very, very promising !!

^1H NMR @ 2-45-1 mixture of crude

2-45-2
2-45-3
2-45-4



So Cbz attached to C(10) OH

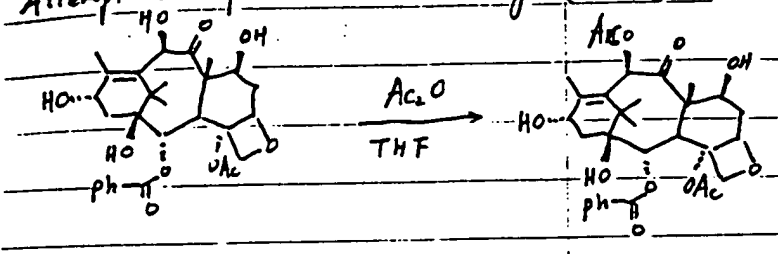
What a surprise ???!

high yield is obtained (≥90%)

Exhibit B (Redacted)

SEP 12 2000 49

Attempt to protect C(10) H by $(CH_3\overset{O}{\overset{\|}{C}})_2O$

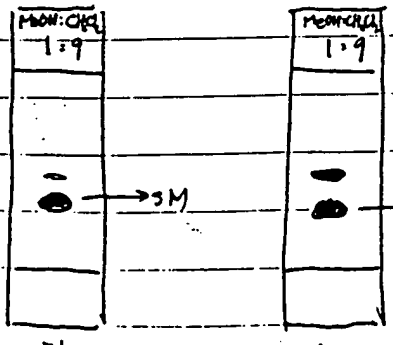


Materials used	FW	AMT
(1) 10DAB	544	3 mg (0.00551 mmol)
(2) Ac_2O	102.09	10 μL (20eqv., 0.11 mmol)
	d = 1.082	
(3) THF		0.5 mL

Procedure: the rxn of 10DAB with $(PhCH_2O\overset{O}{\overset{\|}{C}})_2O$ suggested that similar reaction could take place between 10DAB + Ac_2O

To a solution of 10DAB in THF was added Ac_2O under nitrogen. The reaction mixture was stirred at room temperature and monitored by TLC. TLC indicated the slow process of the rxn and a faint amount of product formed. At this stage it is not clear whether the rxn is proceeding or not. So 100 μL more Ac_2O was added, After 2h, visible rxn was observed, a single spot shows up in high Rf value. After overnight, ≥80% conversion was observed. At this stage, this rxn was stopped by evaporating the solvent away and taken by ^1H NMR

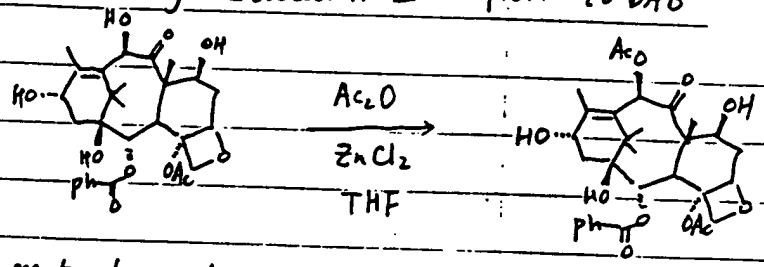
TLC



crude ^1H-NMR 2-49-1 indicated ≥80% Baccatin III + 10DAB + small amount of (7-Ac? 10DAB) (by evaporating the solvent?)

2-49-2 pure Baccatin III.

Generation of baccatin III from 10 DAB

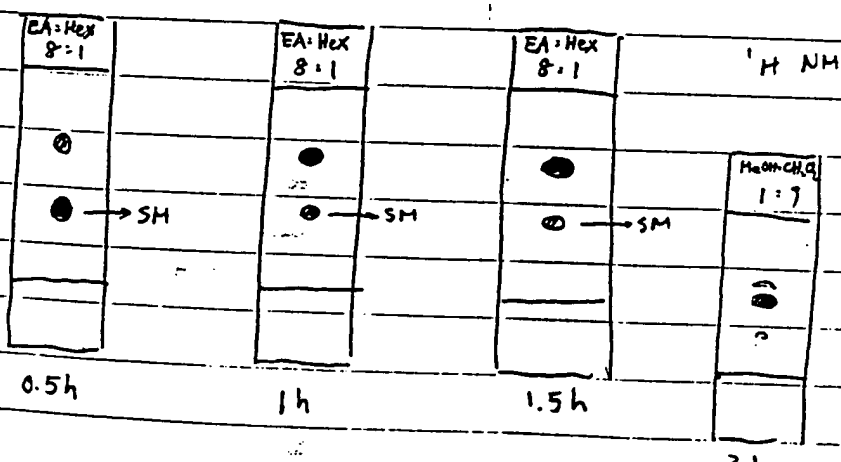


Materials used	FW	AMT
(1) 10 DAB	544	14 mg (0.0258 mmol)
(2) Ac_2O	102.09	1 mL
	d = 1.082	
(3) $ZnCl_2$	136.28	3.5 mg (0.0258 mmol, 1 eq.)
(4) THF		1 mL

Procedure: To a THF solution of 10DAB + $ZnCl_2$ was added Ac_2O under N_2. The solution was stirred at room temperature and monitored by TLC.

actually 18 mg $ZnCl_2$ (may have a little bit of H_2O)

TLC



^1H NMR 2-67-1 crude mixture (too dilute)

2-67-2 crude mixture

Major baccatin III + small amount 7-HO-diacetyl + oxetane ring opened product,

Low temperature experiment is recommended.